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χ-Space Screening of Dermorphin-Based Tetrapeptides through Use of Constrained Arylazepinone and Quinolinone Scaffolds

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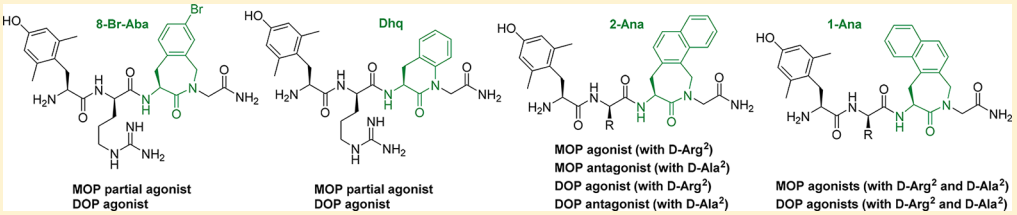
Supporting Information



ABSTRACT: Herein, the synthesis of novel conformationally constrained amino acids, 4-amino-8-bromo-2-benzazepin-3-one (8-Br-Aba), 3-amino-3,4-dihydroquinolin-2-one, and regioisomeric 4-amino-naphthoazepinones (1- and 2-Ana), is described. Introduction of these constricted scaffolds into the *N*-terminal tetrapeptide of dermorphin (i.e., H-Tyr-D-Ala-Phe-Gly-NH_2) induced significant shifts in binding affinity, selectivity, and in vitro activity at the μ- and δ-opioid receptors (MOP and DOP, respectively). A reported constrained μ-/δ-opioid lead tetrapeptide H-Dmt-D-Arg-Aba-Gly-NH_2 was modified through application of various constrained building blocks to identify optimal spatial orientations in view of activity at the opioid receptors. Interestingly, when the aromatic moieties were turned toward the *C*-terminus of the peptide sequences, (partial) (ant)agonism at MOP and weak (ant)agonism at DOP were noticed, whereas the incorporation of the 1-Ana residue led toward balanced low nanomolar MOP/DOP binding and in vitro agonism.

KEYWORDS: *opioid dermorphin ligands, conformational constraints, 4-amino-2-benzazepin-3-one, 3-amino-3,4-dihydroquinolin-2-one, 4-aminonaphthoazepinones*

Nowadays, morphine remains the gold standard analgesic for the treatment of severe to moderate pain. Besides morphine, opioid ligands such as the fentanyl family of painkillers are commonly used in a clinical context. Regrettably, the development of analgesic tolerance and physical dependence, induced by chronic administration of common opioids, limits their long-term use.[1] Despite these severe drawbacks, they remain widely in use to date.

In the search for nonaddictive analgesics with a prolonged duration of action and reduced side effects, a plethora of structural analogues of endo- and exogenous opioid peptides has been explored.[2−4] Many peptidomimetic techniques have been applied in this field, including peptoids,[5] retro-inverso analogues,[6] amide bond isosteres,[7] and macrocyclizations.[8]

Opioid peptides have a common *N*-terminal *message* part and a variable *C*-terminal *address* segment, which is responsible for receptor subtype selectivity (e.g., Figure 1). Receptor selectivity is dependent on the conformational space available to the peptide, and the relative orientation of the Tyr and Phe side chains is indisputably an important feature of opioid peptide pharmacophores.[9,10] Since different receptors may require slightly different side-chain orientations for optimal binding events, receptor (sub)type selectivity can be achieved via introduction of conformationally constrained amino acids. Such conformational constraints provide control over the χ^1- and χ^2-space via favoring, disfavoring, or excluding the *gauche* (−), the *gauche* (+), or the *trans* conformation.[2,4,11−15] In this

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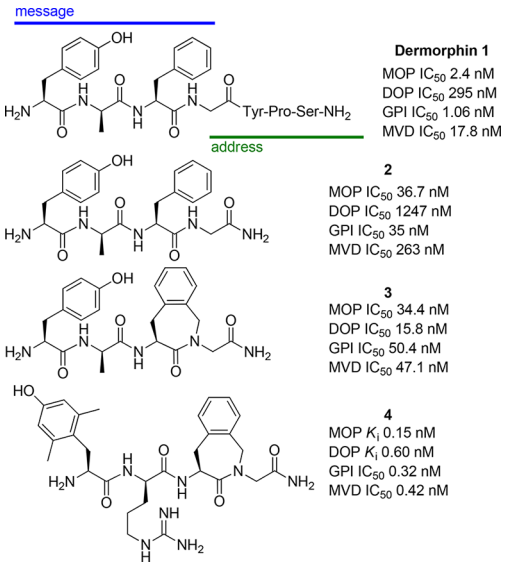

Figure 1. Native dermorphin **1**[9] and truncated analogues H-Tyr-D-Ala-Phe-Gly-NH_2 **2**,[16,17] H-Tyr-D-Ala-Aba-Gly-NH_2 **3**,[18] and H-Dmt-D-Arg-Aba-Gly-NH_2 **4**.[19]

way, useful information about the preferred and bioactive side chain topology can be obtained.[2,13,14]

In particular, the exogenous opioid peptide dermorphin **1** (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) exhibits a high potency and selectivity for the μ-opioid receptor (MOP). *C*-Terminal truncation of sequence **1** revealed that the *N*-terminal tetrapeptide H-Tyr-D-Ala-Phe-Gly-NH_2 **2** (Figure 1) was the minimal segment with full opiate-like activity in vivo.[16,17] To explore the χ-space in **1**, the Phe-Gly dipeptidic segment was previously substituted by the constrained Aba-Gly dipeptidomimetic.[9] Introduction of the 4-amino-1,2,4,5-tetrahydro-3*H*-2-benzazepin-3-one (Aba) scaffold into tetrapeptide **2** at position 3 (i.e., Phe exchanged for Aba) resulted in tetrapeptide **3** (Figure 1).[18] While the Aba-containing ligand (**3**) showed no dramatic change in MOP affinity and activity (determined by the guinea pig ileum (GPI) assay), as compared to the native tetrapeptide sequence, DOP binding and activity significantly increased. DOP agonism was determined via the isolated mouse vas deferens (MVD) test.

Additionally, the replacement of Tyr^1 with the unnatural amino acid 2′,6′-dimethyltyrosine (Dmt) resulted in improved binding to and activity at the opioid receptors.[20] Inspired by [Dmt^1]DALDA (H-Dmt-D-Arg-Phe-Lys-NH_2),[21] D-Ala^2 was replaced by D-Arg^2, to give H-Dmt-D-Arg-Aba-Gly-NH_2 **4** (Figure 1). The latter analogue exhibited excellent MOP/DOP potency and was shown to cross the blood–brain barrier (BBB) after intravenous or subcutaneous administration.[19]

The present study employs novel conformationally constrained Phe-Gly dipeptidomimetics to screen the χ-space of the aromatic Phe^3 side chain within dermorphin's tetrapeptide sequence. Additionally, an extension of the side chain is realized, aimed at improved binding with the opioid receptors but also in an attempt to modulate receptor selectivity and activity. In continuation of our efforts in peptidomimetic design, 4-amino-8-bromo-2-benzazepin-3-one **5** (8-Br-Aba), 3-amino-3,4-dihydroquinolin-2-one **6** (Dhq), and regioisomeric 4-amino-naphthoazepinone **7a–c** (1/2-Ana) scaffolds (Figure 2) were synthesized in solution. Next, these conformationally constrained scaffolds were incorporated into dermorphin-like tetrapeptides using

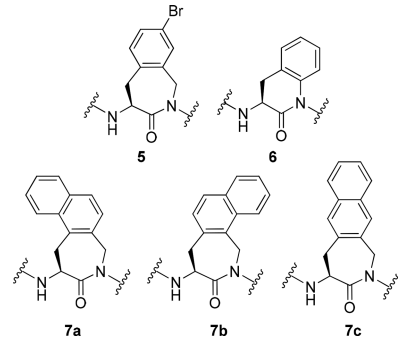

Figure 2. 8-Br-Aba **5**, Dhq **6**, and 1/2-Ana **7a–c** scaffolds as conformationally constrained phenylalanine derivatives.

conventional Fmoc-based solid-phase peptide synthesis (SPPS). The resulting dermorphin sequences were evaluated in vitro using both opioid receptor binding and functional GPI and MVD assays.

The constrained phenylalanine building block Aba (scaffold **5** with H instead of Br) can be prepared via different synthetic pathways, such as attack of an aromatic ring onto a *N*-acyliminium ion intermediate, reductive aminations of *o*-formyl-Phe derivatives, and intramolecular cyclization or even the Ugi-4-component reaction.[22] In order to prepare a diversifiable analogue, the 8-Br-Aba ring was synthesized via either an *N*-acyliminium ion precursor **11** (Scheme 1A) or a reductive amination and subsequent cyclization reaction starting from Phth-2-formyl-L-Phe-OH **15** (Scheme 1B). To access **11**, commercially available 4-Br-L-Phe-OH was *N*-phthaloylated to give Phth-4-Br-L-Phe-OH **8** (Scheme 1A, see the Supporting Information for experimental details). Coupling with ethyl glycinate hydrochloride was achieved by TBTU activation in the presence of Et_3N to give dipeptide Phth-4-Br-L-Phe-Gly-OEt **9** in 75% yield after recrystallization from hot EtOH. Subsequent ethyl ester hydrolysis, to form Phth-4-Br-L-Phe-Gly-OH **10**, was followed by the formation of oxazolidinone **11** by means of paraformaldehyde using a Dean–Stark azeotropic distillation in refluxing toluene. The 7-membered lactam ring in **12** (Phth-8-Br-Aba-Gly-OH) was quantitatively formed via *N*-acyliminium ion cyclization using trifluoromethanesulfonic acid (TFMSA) in dry CH_2Cl_2. Phthaloyl deprotection of **12** by hydrazinolysis and final Fmoc protection resulted in Fmoc-8-Br-Aba-Gly-OH **13**. The enantiomeric ratio was determined with FDAA (Marfey's reagent)[23] derivatization of dephthaloylated **12** (structure not shown). Since LC–MS analysis indicated only one peak corresponding to the desired mass and to make sure that no racemization occurred in this synthetic route, it was decided to couple Phth-8-Br-Aba-Gly-OH **12** with HCl·NH_2-L-Val-O*t*Bu ester via EDC activation in the presence of HOBt. Gratifyingly, a single enantiomer of the resulting tripeptide was observed via LC–MS and ^{1}H NMR analysis (dr ≥99:1).

Alternatively, commercially available 2-cyano-L-Phe-OH was protected as Phth-2-cyano-L-Phe-OH **14** using the same conditions applied for **8**. It was subsequently converted to the corresponding Phth-2-formyl-L-Phe-OH **15** by means of hydrogen and Raney nickel (Scheme 1B).[24] Phth-2-formyl-Phe-OH **15** was converted to Phth-4-Br-2-formyl-Phe-OH **16** using bromoisocyanuric acid monosodium salt (BICA-Na) in concentrated sulfuric acid[25] since other bromination conditions making use of bromine/Lewis acid systems[26] or brominating ionic liquids[27] proved unsuccessful. During the bromination step, the minor regioisomer Phth-6-Br-2-formyl-Phe-OH (structure

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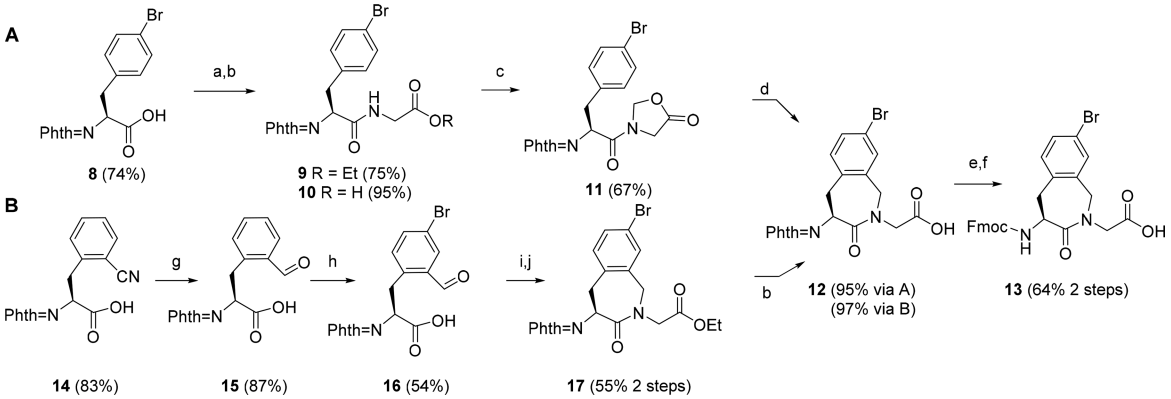
Scheme 1. Synthesis of Fmoc-8-Br-Aba-Gly-OH 13[a]

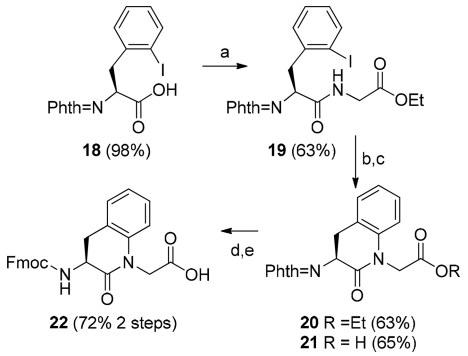


[a]Key: (a) HCl·NH_2-Gly-OEt, TBTU, Et_3N, dry CH_2Cl_2, rt, 16 h; (b) 1 N HCl_{aq}/acetone, 90 °C, 16 h; (c) $(CH_2O)_n$, *p*-TosOH, Dean−Stark apparatus, dry toluene, 115 °C, 5 h; (d) TFMSA, dry CH_2Cl_2, rt, Ar atmosphere, 16 h; (e) $NH_2NH_2{\cdot}H_2O$, EtOH, 90 °C, 2 h; (f) Fmoc-OSu, Na_2CO_3, acetone/H_2O (1:1 v/v), rt, 19 h; (g) H_2 50 psi, RaNi, Py/AcOH/H_2O (1:1:1 v/v), 50 °C, 19 h; (h) BICA-Na, 60% H_2SO_4, rt, 6 h, (Phth-6-Br-2-formyl-Phe-OH separated via RP column chromatography); (i) HCl.NH_2-Gly-OEt, $NaCNBH_3$, dry CH_2Cl_2, rt, 3 h; (j) DCC, CH_3CN, rt, 2 h.

not shown) was also formed (ratio 84:16 based on RP-HPLC peak area) and separated from the desired product **16** by reversed-phase column chromatography and isolated in 54% yield. Reductive amination with ethyl glycinate hydrochloride followed by intramolecular cyclization by means of DCC activation in acetonitrile afforded compound **17**. After ethyl ester hydrolysis, the dipeptide mimetic Phth-8-Br-Aba-Gly-OH **12** was obtained. Considering the overall yield and undetected racemization of pathway A, this route was preferred. The second pathway is, however, more broadly applicable, since pathway A is restricted to α-amino acid use.

Whereas we have recently published the synthesis of 3-amino-3,4-dihydro-1*H*-quinolin-2-ones (Dhq) through regioselective Pd-catalyzed intramolecular cyclization,[28] we propose here another method to access the 3-amino-3,4-dihydroquinolin-2-one **6** (Dhq) core through a key intramolecular Cu(I)-catalyzed Goldberg amidation reaction (Scheme 2).[29−31] Therefore, commercially available 2-I-L-Phe-OH was *N*-phthaloylated to afford Phth-2-I-L-Phe-OH **18** (see the Supporting Information). Subsequently, dipeptide formation was followed by the Cu(I)-catalyzed Goldberg reaction using CuI/*N*,*N*′-dimethylethylenediamine/K_3PO_4 as a catalytic system in dry and degassed toluene. This resulted in a clean conversion to the desired δ-lactam **20** in 63% yield. Ethyl ester hydrolysis gave Phth-Dhq-Gly-OH **21**, and subsequent phthaloyl deprotection-Fmoc protection provided Fmoc-Dhq-Gly-OH **22** in good yield. The enantiomeric ratio was determined via coupling of Phth-Dhq-Gly-OH **19** with HCl.NH_2-L-Val-O*t*Bu. A single enantiomer of the resulting tripeptide was observed via LC−MS and ^{1}H NMR analysis (dr ≥99:1), indicating that no racemization of Phe's original α-carbon occurred during the Cu(I)-catalyzed amidation reaction under the conditions described in Scheme 2.

Scheme 2. Synthesis of Fmoc-Dhq-Gly-OH 22[a]



[a]Key: (a) HCl·NH_2-Gly-OEt, TBTU, Et_3N, dry CH_2Cl_2, rt, 4 h; (b) CuI, *N*,*N*′-dimethylethylenediamine, K_3PO_4, dry and degassed toluene, 120 °C, Ar atmosphere, 67 h; (c) 1 N HCl_{aq}/acetone, 90 °C, 45 h; (d) $NH_2NH_2{\cdot}H_2O$, EtOH, 90 °C, 4 h; (e) Fmoc-OSu, Na_2CO_3, acetone/H_2O (1:1 v/v), rt, 20 h.

Molecular modeling of the *N*-acetyl-*N*′-methylamide tetrapeptide model of dipeptide **6** (i.e., Ac-Dhq-Gly-NHMe) indicated that the lowest energy conformer showed a *trans* conformation along the $C_\alpha-C_\beta$ bond (χ^1 dihedral angle) and was consistent with an (inverse) γ-turn conformation, leading to a pseudo-7-membered ring ($i \rightarrow i \pm 2$) (see the Supporting Information). In contrast, the above-described Aba moieties had previously been shown to prefer extended conformations.[32]

Moreover, 4-aminonaphthoazepinones **7a−c** were synthesized in complete analogy to 8-Br-Aba **5** (cfr. Scheme 1A). Commercially available 1- and 2-L-Nal-OH were *N*-phthaloylated to provide Phth-1/2-L-Nal-OH **23** and **24** (see the Supporting Information). Dipeptide formation followed by ethyl ester hydrolysis gave Phth-1/2-L-Nal-Gly-OH **27** and **28** (Scheme 3). Next, oxazolidinones **29** and **30** were formed in the presence of paraformaldehyde and a catalytic amount of *p*-TosOH. Recrystallization of crude oxazolidinones **29** and **30** from hot toluene resulted in rather low yields (39 and 30%, respectively), and both products **29** and **30** appeared to be unstable during silica gel flash chromatography. Switching to benzene as crystallization medium improved significantly the final yields (72 and 65%, respectively). The 7-membered lactam rings of **31** (1-Ana) and **32a**,**b** (2-Ana) were again accessed via *N*-acyliminium ion cyclization using TFMSA. Despite the higher reactivity of position 1 versus position 3 in naphthyl groups for electrophilic aromatic substitutions, two regioisomers **32a** and **32b** were obtained from the educt Phth-2-L-Nal-oxazolidinone **30** (9:1 ratio based on RP-HPLC peak area). Lowering the reaction temperature from room temperature to, respectively, 0 and −78 °C during the *N*-acyliminium cyclization reaction did not improve the overall conversion into the major 2-Ana

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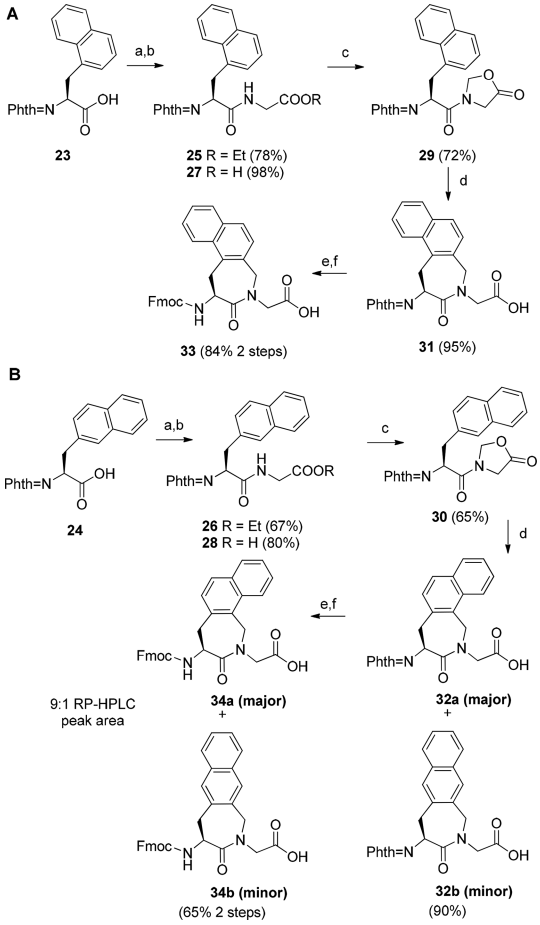
Scheme 3. (A) Synthesis of Fmoc-1-Ana-Gly-OH 33 and (B) Synthesis of Fmoc-2-Ana-Gly-OH 34a,b[a]



[a]Key: (a) HCl·NH_2-Gly-OEt, TBTU, Et_3N, dry CH_2Cl_2, rt; (b) 1 N HCl_{aq}/acetone, 90 °C; (c) $(CH_2O)_n$, *p*-TosOH, Dean−Stark apparatus, dry toluene, 115 °C; (d) TFMSA, dry CH_2Cl_2, rt, Ar atmosphere; (e) $NH_2NH_2.H_2O$, EtOH, 90 °C; (f) Fmoc-OSu, Na_2CO_3, acetone/H_2O (1:1 v/v), rt.

component **32a**. Phthaloyl deprotection and Fmoc protection of both **31** and **32a,b** afforded Fmoc-1-Ana-Gly-OH **33** and the two regioisomers Fmoc-2-Ana-Gly-OH **34a,b** in good yields. Since regioisomers **32a,b** and **34a,b** were inseparable via silica gel column chromatography and preparative RP-HPLC, it was decided to use them as a mixture in Fmoc-based SPPS of the dermorphin tetrapeptides and attempt a separation after peptide assembly.

The conformationally constrained Phe-Gly dipeptidomimetic building blocks **13**, **22**, **33**, and **34a,b** were then used to prepare analogues of the tetrapeptide **4** using standard Fmoc-based SPPS on Rink amide AM resin via DIC/HOBt activation. The tetrapeptide analogues **35**−**41** were evaluated in vitro in order to determine their pharmacological profile. The peptide analogues containing either **34a** or **34b** could be separated by reversed-phase preparative HPLC.

The binding affinities of tetrapeptides **35**−**41** for MOP and DOP receptors were determined by competitive displacement of [^{125}I]-DAMGO and [^{125}I]-Deltorphin II, respectively. The in vitro activity data of peptides **35**−**41** were obtained by measuring the inhibition of electrically induced contractions in the isolated GPI and MVD functional assays (Table 1). Taking into consideration that lead tetrapeptide H-Dmt-D-Arg-[Aba-Gly]-NH_2 **4** possesses K_i values of 0.15 and 0.60 nM for MOP and DOP, respectively, and IC_{50} values of 0.32 nM (GPI) and 0.42 nM (MVD) in the functional assays,[19] data in Table 1 indicate that the insertion of 8-Br-Aba, Dhq and 1/2-Ana constraints at position 3 in the dermorphin sequence did not dramatically change the low nanomolar MOP affinity and all analogues proved good ligands for this receptor (IC_{50} ranging from 0.33 to 2.19 nM). In contrast, DOP affinity was significantly decreased for all tetrapeptides apart from **37**, **38**, and **41**, all possessing the 1-Ana-Gly constraint. These observations were nicely reflected in the GPI and MVD functional assays. Opioid tetrapeptide H-Dmt-D-Arg-[8-Br-Aba-Gly]-NH_2 **35** (DOP IC_{50} 60.7 nM; MVD IC_{50} 8.51 nM) displayed decreased DOP binding affinity and activity, as compared to the Aba-Gly lead peptide **4** (DOP K_i 0.60 nM; MVD IC_{50} 0.42 nM),[19] while MOP affinity was retained (MOP IC_{50} 0.76 nM). This observation indicates that DOP tolerates the bromine substituent less than MOP does in view of efficient binding. Similarly, introduction of the Dhq-Gly dipeptidomimetic to give H-Dmt-D-Arg-[Dhq-Gly]-NH_2 **36** resulted in dramatically decreased DOP binding affinity (DOP IC_{50} 630 nM) and activity (MVD IC_{50} 90.5 nM). This could indicate that the conformation induced by the γ-turn promoting δ-lactam constraint in **36** was not beneficial toward DOP binding and receptor activation and/or means that beneficial interactions with the Dhq aromatic ring are lost in this type of ligand.

Table 1. In Vitro Opioid Receptor Affinity and Activity of Dermorphin Tetrapeptides 35−41

no.	compd	GPI[a] (IC_{50}, nM)	MVD[a] (IC_{50}, nM)	MOP[b] (IC_{50}, nM)	DOP[b] (IC_{50}, nM)	selectivity IC_{50} DOP/IC_{50} MOP
35	H-Dmt-D-Arg-[8-Br-Aba-Gly]-NH_2	22.0 ± 2.4[c]	8.51 ± 0.70	0.76 ± 0.49	60.7 ± 13.7	80
36	H-Dmt-D-Arg-[Dhq-Gly]-NH_2	33.7 ± 3.6[d]	90.5 ± 14.4	1.62 ± 0.03	630 ± 65.0	389
37	H-Dmt-D-Arg-[1-Ana-Gly]-NH_2	0.252 ± 0.014	1.42 ± 0.18	0.33 ± 0.01	1.00 ± 0.45	3
38	H-Dmt-D-Ala-[1-Ana-Gly]-NH_2	1.13 ± 0.13	0.86 ± 0.16	0.39 ± 0.07	2.07 ± 0.30	5
39	H-Dmt-D-Arg-[2-Ana-Gly]-NH_2 major	21.4 ± 1.9	114 ± 7.0	2.19 ± 0.70	153 ± 12.0	70
40a	H-Dmt-D-Ala-[2-Ana-Gly]-NH_2 major	2.88 ± 0.10 (K_e, antagonist)	306 ± 12.0 (K_e, antagonist)	1.78 ± 0.40	167 ± 38.0	94
40b	H-Dmt-D-Ala-[2-Ana-Gly]-NH_2 minor	5.53 ± 1.12 (K_e, antagonist)	360 ± 58.0 (K_e, antagonist)			
41	H-Dmt-*N*Me-D-Ala-[1-Ana-Gly]-NH_2	0.43 ± 0.06	1.09 ± 0.04	0.35 ± 0.04	1.47 ± 0.04	4

[a]The GPI functional assay is representative of MOP activation, whereas the MVD is a DOP receptor-representative assay. [b]Binding affinities of compounds for MOP and DOP opioid receptors were determined by competitive displacement of [^{125}I]-DAMGO (MOP-selective) and [^{125}I]-Deltorphin II (DOP-selective) from HEK293 cells expressing MOP or DOP. [c]IC_{25}, partial agonist. [d]IC_{35}, partial agonist.

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However, both tetrapeptides **35** and **36** showed only partial agonist activity at MOP in the GPI functional assay, and hence, full MOP agonism was lost through these modifications. Interestingly, incorporation of the regioisomeric 4-amino-naphthoazepinones (Figure 2 and Scheme 3) resulted in distinct biological profiles (Table 1): H-Dmt-D-Arg-[1-Ana-Gly]-NH_2 **37** and H-Dmt-D-Ala-[1-Ana-Gly]-NH_2 **38** showed excellent low nanomolar MOP and DOP binding affinities, while DOP affinity was lost for H-Dmt-D-Arg-[2-Ana-Gly]-NH_2 **39** (DOP IC_{50} 153 nM) and its D-Ala^2 analogues, H-Dmt-D-Ala-[2-Ana-Gly]-NH_2 **40a,b** (DOP IC_{50} 167 nM for **40a**; **40b** was not tested).

While the incorporation of the 1-Ana scaffold in **37** and **38** led to in vitro agonism at MOP and DOP, the 2-Ana-constrained peptides **39** and **40a,b** showed moderate MOP/DOP agonism (**39**) and even potent MOP/weak DOP antagonism (**40a,b**). Similarly to the recently reported cyclodals (cyclic Dmt^1[DALDA] analogues, structures not shown),[33] MOP antagonists are of key interest for reversing morphine-induced, centrally mediated analgesia in the treatment of opioid abuse and overdose. From these data, it seems that the 1- versus 2-Ana-Gly dipeptidomimetics are excellent tools to (i) examine the importance of the relative side chain orientations of key pharmacophore aromatic residues and (ii) modulate receptor subtype recognition and activation. Since H-Dmt-*N*Me-D-Ala-[Aba-Gly]-NH_2 is reported to be a superpotent opioid,[34] ligand **38** was *N*-methylated at D-Ala^2 to afford H-Dmt-*N*Me-D-Ala-[1-Ana-Gly]-NH_2 **41** by coupling of commercially available Fmoc-*N*Me-D-Ala-OH. *N*-Methylation of the peptide backbones is a powerful strategy to enhance overall bioavailability, improve metabolic stability toward protease activity, and differentiate receptor subtype selectivity via conformational modulation.[35] H-Dmt-*N*Me-D-Ala-[1-Ana-Gly]-NH_2 **41** showed excellent low nanomolar MOP (IC_{50} 0.35 nM) and DOP (IC_{50} 1.47 nM) affinities. These binding affinities were translated into improved functional activities, as determined in the GPI (IC_{50} 0.43 nM) and MVD (IC_{50} 1.09 nM) tissue bioassays. Compound **41** is also the most hydrophobic analogue among the ligands encompassing the [1-Ana-Gly] constraint, based on RP-HPLC retention times (see the Supporting Information). Therefore, the direct comparison of structures **37**, **38**, **41**, and a reference tetrapeptide H-Dmt-D-Arg-[Aba-Gly]-NH_2 **4**,[19] in terms of in vivo antinociception, will provide insight into the importance of hydrophobicity for eventual in vivo analgesia. In a previous report, we indeed identified hydrophobicity to be a key determinant for activity, rather than the presence of a conformational azepinone constraint.[36]

In conclusion, the insertion of constricted 4-amino-8-bromo-2-benzazepin-3-one **5** (8-Br-Aba), 3-amino-3,4-dihydroquinolin-2-one **6** (Dhq), and regioisomeric 4-amino-naphthoazepinones **7a−c** (1/2-Ana) dipeptidomimetics into the optimized dermorphin tetrapeptide lead sequence has resulted in compact and high affinity MOP/DOP opioid receptor ligands. Whereas insertion of the conformationally constrained 8-Br-Aba-Gly, Dhq-Gly, or 2-Ana-Gly dipeptides resulted in decreased DOP recognition, application of the regioisomeric 1-Ana-Gly building block led toward excellent low nanomolar MOP and DOP binding affinities and in vitro functional activities. The described cyclic constrained aromatic amino acids can be regarded as additional tools to modulate receptor selectivity and activity, but they also provide a way to enhance proteolytic stability and bioavailability of lead peptides. Bulky Nal residues often provide a means of modulating receptor selectivity.[37] The current building blocks present an additional feature to control χ dihedral angles, thus presenting specific ligand topologies. The present study clearly showcases the advantages of such constrained residues for improving ligand potency and opioid receptor selectivity. It is expected that these building blocks will find application in other biologically active peptides.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00347.

Complete experimental details along with the characterization of the synthesized compounds (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

Aba, 4-amino-1,2,4,5-tetrahydro-3*H*-2-benzazepin-3-one; Ana, 4-amino-naphthoazepinone; BBB, blood−brain barrier; DALDA, H-Tyr-D-Arg-Phe-Lys-NH_2; Dhq, 3-amino-3,4-dihydroquinolin-2-one; DCC, 1,3-dicyclohexylcarbodiimide; DIC, 1,3-diisopropylcarbodiimide; Dmt, 2′,6′-dimethyltyrosine; DOP, δ-opioid receptor; EDC·HCl, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride; FDAA, 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide; GPI, guinea pig ileum; HEK, human embryonic kidney; HOBt, 1-hydroxybenzotriazole; IC_{50}, concentration needed to replace 50% of a receptor-bound ligand; MOP, μ-opioid receptor; MSB, methyl 2-((succinimidooxy)carbonyl)benzoate; MVD, mouse vas deferens; $NaCNBH_3$, sodium cyanoborohydride; SPPS, solid-phase peptide synthesis; RaNi, Raney Nickel; TBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; TFMSA, trifluoromethanesulfonic acid

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